THE RESPIRATORY FUNCTION OF THE BLOOD OF URECHIS CAUPO

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Urechis caupo is an echiuroid worm inhabiting sandy mud flats in the estuaries of the coast of California. Its characters and habits have been described by Fisher and MacGinitie (1928). Because of its large size, the simple nature of its circulatory and respiratory systems, and the fact that its cœlomic fluid is voluminous and contains abundant red blood corpuscles, it affords unusually suitable material for the study of respiratory problems. The present paper contains a description of those properties of the blood of *Urechis* which are of importance in respiration, together with certain observations designed to evaluate the significance of these properties.

The material used for these studies was collected in the Elkhorn Slough, a tributary of Monterey Bay. The authors wish to express their indebtedness to Dr. MacGinitie for assistance in procuring the animals and to Professor Fisher for the many courtesies received while they were at the Hopkins Marine Station.

I. The Blood of Urechis

Fisher and MacGinitie (1928) state that the cœlom is filled with bright red blood, the pigment being lodged in subcircular cells, about 0.025 mm. in diameter, which readily distort when crowded. There are also very numerous amœboid cells which when aggregated are yellow in color. We have found the color of the blood to vary, being frequently of a dull brownish-red color; less often, and particularly in smaller specimens, of a bright scarlet resembling the blood of vertebrates. A volume of 15 or 20 cc. may be secured from a single specimen.

The Blood

The plasma does not clot, and when separated from the cells is a pale yellow color; not infrequently it may be tinged with the corpuscular pigment. Under microscopic examination the cytoplasm of the corpuscles appears yellow and is seen to be filled with small, highly refractive granules. In addition, there are many granules of a brown pigment

in the corpuscles of some specimens. The occurrence of this pigment is variable and it may be nearly lacking in those specimens whose blood appears scarlet rather than brownish-red. In the center of the corpuscle is a clearer area, which, on staining, proves to be a small nucleus. The cœlomic cells of *Urechis chelensis* described by Seitz (1907) are apparently similar, being nucleated and containing yellow pigment granules (in preserved material).

TABLE I

Spectrometric Data of Urechis Hemoglobin

Wave Length	Oxyhemo-globin	Reduced Hemoglobin	Wave Length	Oxyhemo-globin	Reduced Hemoglobin
$m\mu$	E	E	$m\mu$	E	E
450.4	0.394	0.495	566.6	0.221	0.299
460.5	0.280	0.225	567.6	0.223	
470.6	0.226	0.169	569.7	0.245	0.273
480.7	0.189	0.160	571.7	0.285	
490.8	0.179	0.164	573.7	0.297	
500.9	0.174	0.176	575.7	0.309	
511.0	0.172	0.197	576.7	—-	0.238
516.1	0.180		577.8	0.310	
521.1	0.192	0.218	578.9	0.297	
523.2	0.206		581.8	0.267	0.204
525.2	0.215		583.8	0.214	
527.2	0.231		585.8	0.168	
529.3	0.244		586.9	—	0.171
531.3	0.264	0.245	587.9	0.131	
533.3	0.276		589.9	0.102	
535.3	0.291		591.9	0.082	0.145
537.3	0.299		597.0	0.055	
539.3	0.306		602.0	0.042	0.096
541.4	0.312	0.277	612.1	0.032	0.073
543.4	0.310		622.2	0.025	0.064
545.4	0.292		632.4	0.022	0.059
546.4	—	0.298	642.5	0.024	0.056
547.4	0.276		652.6	0.009	0.049
549.5	0.255		662.7	0.016	0.046
551.5	0.243	0.313	672.8	0.004	0.038
555.5	0.214		682.9	0.008	0.036
556.5	—	0.313	693.0	0.003	0.028
559.6	0.197				
561.6	0.197	0.312			
563.6	0.202				
565.6	0.211				

The corpuscles appear to be surrounded by a strong membrane. On dilution of blood with distilled water, the cells swell but do not burst. Upon applying pressure to the coverslip when in this condition the membrane ruptures and the contents may be seen to flow out through a localized opening. The granules in the swollen corpuscles are in active Brownian movement, suggesting a fluid state of the interior of the cell.

In a one per cent saponin solution the cells swell, the granules remaining confined to the previous volume of the cells and appearing surrounded by a clear region. After a few minutes the membrane spontaneously ruptures and the granules flow out through the localized opening.
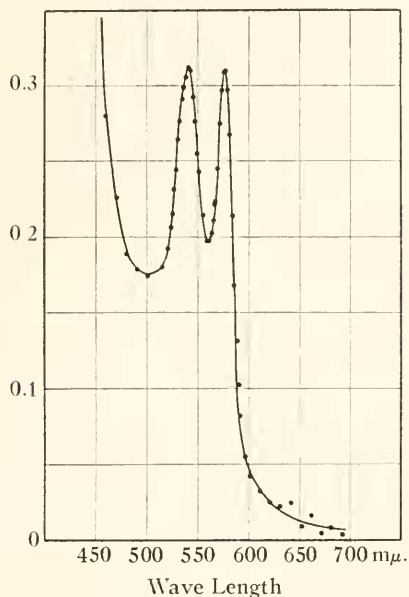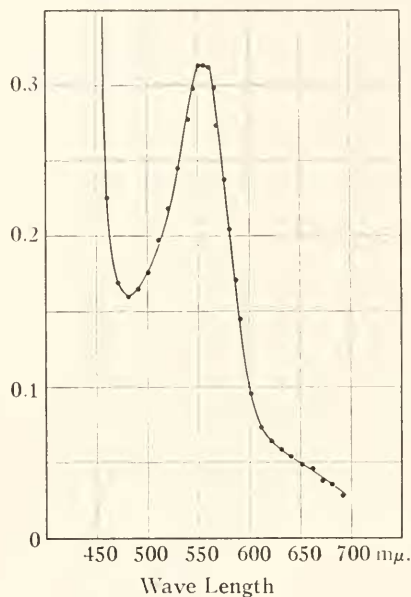

FIG. 1. Absorption spectrum of oxygenated hemoglobin of *Urechis caupo*. Ordinates: extinction cœfficient of solution of unknown concentration.
        FIG. 2. Absorption spectrum of reduced hemoglobin of *Urechis caupo*. Ordinates: extinction cœfficient of solution of same concentration as that shown in Fig. 1.

The Respiratory Pigment

That the corpuscles of *Urechis* contain hemoglobin is indicated by the spectroscopic examination of laked blood. Typical hemoglobin crystals may be obtained by allowing laked corpuscles to dry under a coverglass. For spectrophotometric examination a solution of hemoglobin was prepared by laking one cc. of corpuscles separated by centrifugation with 11 cc. of distilled water containing three drops of ether. To this solution was added 8 cc. of 4M ammonium sulfate brought to ca. pH 8 by the addition of ammonia. From this solution the corpuscular debris was filtered off and one volume of the filtrate diluted with four volumes of water. Filtration was repeated. The filtrate so obtained contained the original corpuscular content of hemoglobin diluted 1 : 100 and in the presence of 0.8 molar ammonium sulfate at a pH of approximately 8.

One sample of this solution was employed for measuring the absorption spectrum of oxyhemoglobin; another specimen was reduced by equilibration with hydrogen and used to obtain the spectrum of reduced hemoglobin. The solutions were perfectly clear. The measurements were made with a König-Martens spectrophotometer within five hours of the completion of the preparations. The length of the column of fluid was 3.3 cm. The extinction cœfficients of these solutions, estimated for one cm. length, are recorded in Table I and illustrated graphically in Figs. 1 and 2. In Table II are recorded the wave length of maximum density in the α and β bands and the wave length of minimum absorption between these bands as obtained by various workers with various hemoglobins.

TABLE II

Spectrometric Characteristics of Various Oxyhemoglobins

Species	Wave Length of Maximum Absorption		Wave Length of Minimal Absorption between α and β Bands	Ratio of Extinction Coefficients at Maximum β and Minimum between α and β	Observer
	α Band	β Band			
	$m\mu$	$m\mu$	$m\mu$		
Dog and horse....	575.6	540.4	558.1	>1.60	Hári (1917)
Dog.......	575–577	539–542	560.*	1.63	Kennedy (1926–27)
Horse.....	578.2	540.4	562.5*	1.58*	Vlès (1923)
Arenicola..	576.0	540.0	560.*	1.53*	Vlès (1923)
Marphysa.	578.0	540.0			Vlès (1923)
Cucumaria	579.	542.7	558.		Van der Lingen and Hogben (1928)
Urechis....	577.	542.	561.	1.58	

* Estimated from published data of observer.

These values for *Urechis* hemoglobin agree more closely on the whole with those found for the hemoglobin of the worm *Marphysa* and the holothurian *Cucumaria* and with Vlès' measurements of horse hemoglobin than with this author's data for *Arenicola*. The values for horse hemoglobin obtained by Vlès, from which he concluded that *Arenicola* hemoglobin differed from horse hemoglobin, do not agree with the values obtained by Hári and Foster for the mammalian pigment, which are very similar to Vlès' values for *Arenicola*. The shape of the absorption curve for *Urechis* does not agree exactly with the data recorded for mammalian oxyhemoglobin, particularly in the region about 510 mμ. Discrepancies in the shape of the curves may be attributed to the

presence of methemoglobin in the solutions, as Hári has pointed out; a fact which makes the direct comparison of the curves difficult. The data leave no doubt that the pigment of the *Urechis* blood is a hemoglobin, but the spectrometric evidence regarding the specificity of the hemoglobin cannot safely be interpreted.

TABLE III

Cell Volume and Oxygen Capacity of Urechis Blood

Specimen	Volume Red Cells	Oxygen Content	Oxygen Combined	Oxygen Combined per 100 cc. Cells
	per cent	*volumes per cent*	*volumes per cent*	*cc.*
3	36.6	—	—	—
4	26.4	—	—	—
7	40.3	6.30	5.80	14.4
		7.22	6.72	16.7
8	37.6	5.83	5.33	14.2
		5.70	5.20	13.8
		5.77	5.22	13.9
9	18.3	2.85	2.35	12.8
		2.87	2.37	12.9
10	35.3	5.72	5.22	14.8
		5.53	5.03	14.2
12	23.8	4.64	4.14	17.4
		4.36	3.86	16.2
		4.24	3.74	15.7
13	19.5	3.89	3.39	17.4
		3.70	3.20	16.4
14	23.2	2.90	2.40	10.3
		2.83	2.33	10.0
		2.66	2.16	9.3
15	28.6	4.53	4.03	14.1
		4.30	3.80	13.3
16	32.0	4.54	4.04	12.6
		4.78	4.28	13.4
21	—	3.70	3.20	—
20	—	4.43	3.93	—
		4.50	4.00	—
23	—	4.09	3.59	—
	—	4.05	3.55	—

Hemoglobin appears to occur in the musculature of *Urechis*, particularly in that of the foregut, or crop. In this structure, which is in a thin muscular tube of a bright pink color, the spectrum of oxyhemoglobin can be beautifully demonstrated with the microspectroscope. If the preparation is covered with a coverglass, the spectrum soon changes to that of reduced hemoglobin, except near the edges, where the oxyhemoglobin bands persist. Because of the absence of capillaries in this preparation it should form very advantageous material for the study of the function and properties of muscle hemoglobin.

The Quantity of Corpuscles and Hemoglobin in the Blood

The red corpuscles occupy from 18 to 40 per cent of the total volume of the blood when separated with the hematocrit (Table III). A gray layer of rather variable volume containing sperm or eggs and other cells separates between the red cells and the plasma. The oxygen content of the blood equilibrated with air was determined with the Van Slyke constant volume blood gas apparatus, using one cc. samples, and varies between two and six volumes per cent. Special care was taken to stir the blood before sampling because of the rapid rate at which the large corpuscles settle out. A one per cent saponin solution was used as laking reagent. These values are recorded in Table III and may be compared with the values found for other worms and other invertebrates containing hemoglobin in Table IV.

TABLE IV

Oxygen Content of Blood of Worms and Other Invertebrates
(equilibrated with air)

Species	Oxygen Content	Pigment	Occurrence	Observer
Urechis caupo	2.66–7.22	Hemoglobin	in corpuscles	
Glycera siphonostoma	2.58–3.03	Hemoglobin	in corpuscles	Winterstein (1909)
Arenicola sp.	5.70–8.70	Hemoglobin	in solution	Fox (1926) after Barcroft and Barcroft (1924)
Cardita sulcata	1–2	Hemoglobin	in solution	Winterstein (1909)
Pectunculus violaceus	1–2	Hemoglobin	in solution	Winterstein (1909)
Spirographis	8.40–10.0	Chlorocruorin	in solution	Fox (1926)
Siphunculus nudus	ca. 2	Hemerythrin	in corpuscles	Winterstein (1909)

It is commonly believed that the inclusion of the respiratory pigments within corpuscles has made possible the superior oxygen capacity of the blood of vertebrates. This possibility does not appear to have been realized in the invertebrate stage of development, for *Arenicola* and

Spirographis, which carry their respiratory pigments in solution, have a greater oxygen content than *Urechis* and the other invertebrate forms in which oxygen is transported in blood corpuscles.

The concentration of hemoglobin in the corpuscles of *Urechis* appears to be much less than is the case in vertebrates. In Table III is recorded the estimated oxygen combined per 100 cc. of red corpuscles —allowance being made for 0.50 volumes per cent of oxygen assumed to be present in solution. The oxygen-combining power of the cells varies from about ten to seventeen volumes per cent. Drastich (1928) finds the following values for the hemoglobin content of the cells of vertebrates:—various mammals 29.5 to 34; various birds 29.54; *Rana esculenta* 24.85; carp 26.02 grams per 100 cc. corpuscles. Assuming the *Urechis* hemoglobin to have the same oxygen-combining power per unit weight as mammalian hemoglobin (one volume per cent oxygen capacity corresponding to 0.746 grams of hemoglobin per 100 cc.), *Urechis* corpuscles are estimated to contain 7.5 to 12.7 grams of hemoglobin per 100 cc. of cells. The *Urechis* corpuscle is then about one-third as effective in transporting oxygen as those of the vertebrates. It is to this fact rather than to a deficiency in the number of corpuscles that the low oxygen capacities of the blood are principally due.

TABLE V

Data on Equilibrium of Oxygen with Urechis Blood

Carbon Dioxide Pressure	Oxygen Pressure	Oxygen Content	Oxygen Dissolved	Oxygen Combined	Saturation
mm. Hg	*mm. Hg*	*volume per cent*	*volume per cent*	*volume per cent*	*per cent*
8.64	5.98	0.63	0.192	0.44	19.1
9.78	8.50	0.87	0.027	0.84	36.6
8.61	12.15	1.15	0.039	1.11	48.3
7.90	16.35	1.46	0.053	1.41	61.3
7.54	23.08	1.88	0.074	1.81	78.8
10.87	41.85	2.09	0.135	1.95	84.8
6.54	48.15	2.15	0.155	1.99	86.6
6.11	72.61	2.32	0.234	2.09	90.9
7.56	87.21	2.36	0.281	2.08	90.4
air	air	2.90	0.50	2.40	
		2.83	0.50	2.33	
		2.66	0.50	2.16	
				—	
				av. 2.30	100

Iron Content of Blood

Attempts to estimate the iron content of the blood by the method of Hall and Gray (1929) yielded rather discordant results. The values obtained were always of the order expected from the oxygen capacity of the samples.

The Equilibrium of Oxygen with the Blood

The oxygen dissociation curve of the whole blood has been determined using the Van Slyke constant volume apparatus for blood gas estimations and the Haldane analyzer for measuring the composition of the gas with which the blood has been equilibrated. Equilibration was carried out upon 3 cc. of blood enclosed in 250 cc. tonometers rotated for 20 minutes in a water bath at 19° C. Analyses were made immediately after equilibration in fear that the metabolism of the cells might alter the gaseous content were the samples allowed to stand. The carbon dioxide pressure was maintained approximating that obtaining in the blood *in vivo;* about seven millimeters. The data are recorded in Table V.

In estimating the combined oxygen from the oxygen capacity it is assumed that blood in equilibrium with air dissolves 0.5 volumes per cent oxygen, the solubility at lower oxygen pressures being proportional in accordance with Henry's law. The oxygen dissociation curve is plotted in Fig. 3.
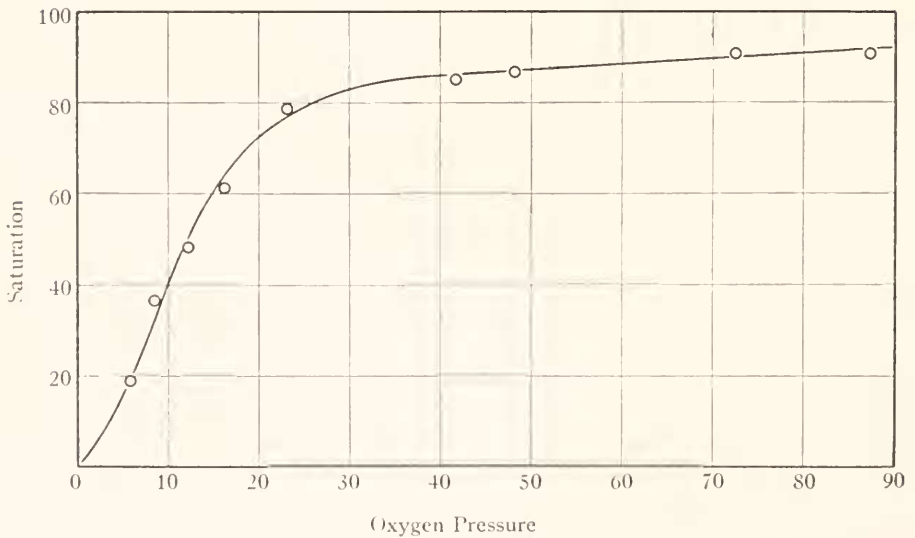

FIG. 3. Oxygen dissociation curve of blood of *Urechis caupo.* Temperature 19° C. For data see Table V. Ordinates: percentage of saturation; abscissæ: partial pressure of oxygen in mm. Hg.

The Effect of Carbon Dioxide upon the Oxygen Dissociation Curve

Samples of blood have been equilibrated with oxygen in the presence of carbon dioxide at pressures varying from 0.54 to 92 mm. Hg. The temperature of equilibration was 19° C. The results are recorded in Table VI. From this data, p_{50}, the oxygen pressure at which the blood would have been half saturated with oxygen has been calculated, assuming the curves to have the same shape as that drawn in Fig. 3.

TABLE VI

Data on Equilibrium of Blood with Oxygen at Various CO_2 Pressures

Specimens	Carbon Dioxide Pressure	Oxygen Pressure	Oxygen Content	Oxygen Dissolved	Oxygen Combined	Saturation	p_{50}
	mm. Hg	mm. Hg	vol. per cent	vol. per cent	vol. per cent	vol. per cent	mm. Hg
	0.54	10.40	1.96	0.03	1.93	49	10.6
	0.94	14.89	2.62	0.04	2.58	66	10.7
	8.60	7.10	2.16	0.02	2.14	55	6.3
Urechis No. 15	8.82	14.12	2.60	0.04	2.56	66	10.1
	19.60	8.40	2.25	0.12	2.23	57	7.2
	19.80	16.25	2.68	0.05	2.63	67	11.3
	29.40	12.55	2.20	0.04	2.16	55	11.2
	air	air	4.53	0.50	4.03	103.3	—
			4.30	0.50	3.80	97.4	—
	0.62	8.38	1.74	0.03	1.71	41.	9.9
	0.71	10.80	1.95	0.03	1.92	46.	11.7
	0.76	9.56	1.96	0.03	1.93	47.	10.1
Urechis No. 16	1.26	9.44	1.77	0.03	1.74	42.	11.1
	77.0	9.08	2.14	0.03	2.11	51.	9.0
	92.0	11.30	2.34	0.04	2.30	55.	10.1
	air	air	4.54	0.50	4.04	97.	—
			4.78	0.50	4.28	103.	—

These values, recorded in the last column of Table VI, make it appear that the affinity of the blood for oxygen is not influenced to a detectable degree by the pressure of carbon dioxide within the ranges of pressure examined. In this regard the blood of *Urechis* differs from that of most vertebrates and from that of *Arenicola*. In the latter form Barcroft and Barcroft (1924) found the typical effect of hydrogen ion concentration upon the oxygen dissociation curve. Recently Dill and Edwards (1931) have observed that in the blood of the elasmobranch, *Raia oscillata*, the effect of carbon dioxide upon the oxygen dissociation curve is absent or nearly so.

The Effect of Temperature upon the Oxygen Dissociation Curve

Oxygen dissociation curve data obtained from the same specimen of blood have been secured at two temperatures, 22° C. and 34.5° C. (Fig. 4). The carbon dioxide tension was about 12 mm. in both cases.
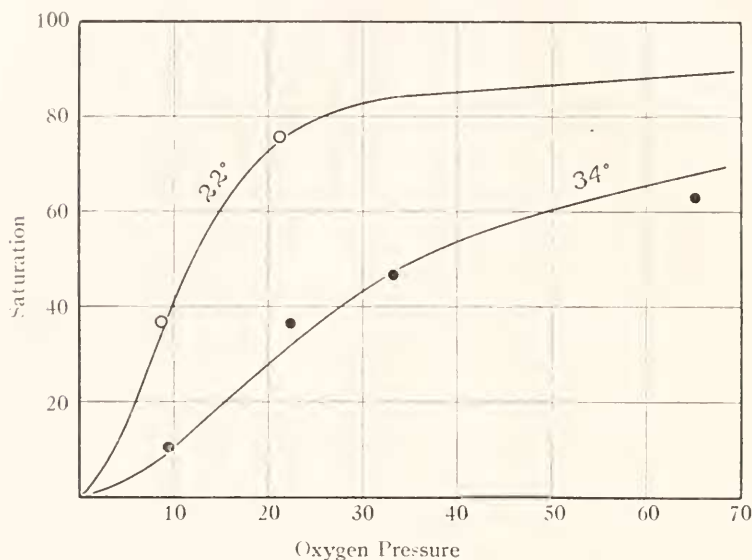

FIG. 4. Oxygen dissociation curves of blood of *Urechis caupo*, equilibrated at temperatures of 22° C. and 34° C. Ordinates: percentage of saturation; abscissæ: partial pressure of oxygen in mm. Hg.

The curve drawn through the data obtained at 22° C. is identical with that in Fig. 2 obtained from another sample of blood at 19° C. At 34° C. the points lie well to the right. The data are insufficient to warrant any conclusion with regard to the shape of the curve at the higher temperature, but it is clear that the temperature effect upon the oxygen equilibrium is large and of the same direction and order observed in vertebrate hemoglobin (Brown and Hill, 1923; Macela and Seliškar, 1925).

The Equilibrium of Carbon Dioxide with the Blood

Table VII presents the data obtained by equilibrating *Urechis* blood against various mixtures of carbon dioxide in air at 18.5° C. The analyses were made with the Van Slyke apparatus and the Haldane analyser. The oxygen capacity of the blood employed corrected for dissolved oxygen was 3.9 volumes per cent.

In order to facilitate comparison of the *Urechis* blood with that of

TABLE VII

Data on Equilibrium of Carbon Dioxide with Urechis Blood

Carbon Dioxide Pressure	Carbon Dioxide Content	Carbon Dioxide Dissolved (H_2CO_3)	Carbon Dioxide Combined ($BHCO_3$)	$\log \dfrac{(BHCO_3)}{(H_2CO_3)}$
mm. Hg	*vol. per cent*	*vol. per cent*	*vol. per cent*	
0.9	3.32	0.09	3.23	1.597
3.3	6.13	0.35	5.78	1.217
7.22	8.90	0.76	8.14	1.029
12.40	11.00	1.30	9.70	0.873
22.0	14.15	2.31	11.84	0.710
47.2	19.15	4.96	14.19	0.456

other animals, and for the comparison of various experiments with this species it is convenient to relate the data to the logarithm of the ratio of combined ($BHCO_3$) to free (H_2CO_3) carbonic acid. This function changes approximately in proportion to the hydrogen ion concentration, which may be obtained by adding the appropriate pK value. Moreover, the total buffer value of the blood is also dependant upon this function. The quantity of carbon dioxide dissolved in the blood or present as H_2CO_3 (free carbonic acid) has been estimated assuming α, the number of cubic centimeters of CO_2 dissolved in one cubic centimeter at a pressure of 760 mm. Hg, to be 0.80. This value is slightly less than the value 0.827 given by Bohr (1897) for two per cent NaCl at 18° C.

The concentration of combined carbonic acid ($BHCO_3$) is obtained by subtracting the free carbonic acid (H_2CO_3) from the total carbonic acid. The estimated values of these quantities are included in the table.

The total buffer value of blood, β, is defined by the equation

$$\beta = \frac{-\triangle (BHCO_3)}{\triangle \log \dfrac{(BHCO_3)}{(H_2CO_3)}}$$

In Fig. 5 the values of ($BHCO_3$) are plotted against $\log \dfrac{(BHCO_3)}{(H_2CO_3)}$.

Throughout a considerable range the points fall about a straight line, indicating as in the case of mammalian blood that the buffer value is constant. The value of β is given by the slope of this line and is 11 volumes per cent (or 0.49 milliequivalents per liter).

It will be shown subsequently that the plasma of *Urechis* possesses little or no buffer value. Is the total buffer value of the *Urechis* blood adequately accounted for by the quantity of hemoglobin in the corpuscles? In this specimen of blood the oxygen capacity was 3.9 volumes per cent. The buffer value per equivalent of hemoglobin is given

by β, 3.9 = 2.82. This value is intermediate between the buffer values of oxygenated and reduced hemoglobin as it occurs in the cells of the blood of man and of the crocodile, the extreme values being 2.4 for
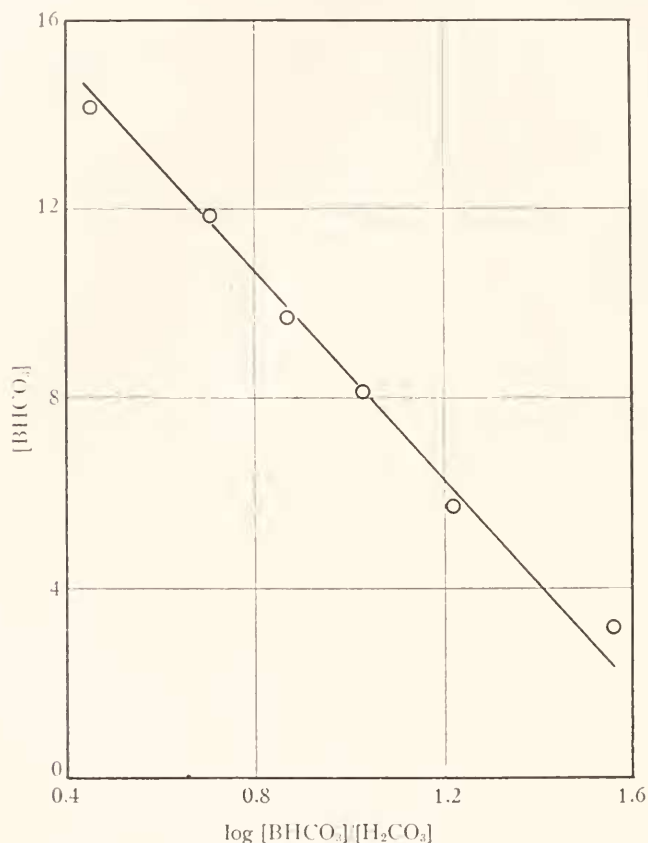

Fig. 5. Relation of combined carbonic acid (BHCO₃), to log $\frac{(BHCO_3)}{(H_2CO_3)}$ for blood of *Urechis caupo*. Temperature 19° C. For data see Table VII.

reduced crocodile blood at 29° and 3.47 for oxygenated blood of this species (Dill and Edwards, 1931). The concentration of hemoglobin appears sufficient to account for the total buffering effect of *Urechis* blood.

The effect of oxygenation and reduction upon the carbon dioxide-combining power of blood is considered to be directly related to the reciprocal effect of carbon dioxide (or hydrogen ion concentration) upon the oxygen dissociation constant of hemoglobin (Henderson, 1928, Chapter IV). We have shown in the case of *Urechis* that the latter

is uninfluenced by the quantity of carbon dioxide present, and it is consequently interesting to inquire whether reduced blood possesses the same carbon dioxide-combining power as oxygenated blood. Dill and Edwards (1931) state that both effects are absent or nearly so in the blood of the skate, *Raia oscillata.*

As the effect is proportional to the concentration of hemoglobin, it may be expected to be small in any case. In order to facilitate the experiments, the blood of about ten animals was mixed and the corpuscles separated with the centrifuge. A small quantity of plasma was mixed with the corpuscles, yielding a solution containing 78 per cent red blood corpuscles and having an oxygen capacity of 10.7 volumes per cent. Were the *Urechis* hemoglobin similar to mammalian hemoglobin the reduced solution should combine four or five volumes per cent more carbon dioxide than the oxygenated serum when $\log \dfrac{(BHCO_3)}{(H_2CO_3)}$ is 0.8. Table VIII shows the result of equilibrating samples of this concentrated blood with air and with hydrogen containing about twenty-two mm. pressure of carbon dioxide. The quantity of oxygen found in the

TABLE VIII

Data on Carbon Dioxide Equilibrium in Oxygenated and Reduced Blood

Oxygen Pressure	Carbon Dioxide Pressure	Carbon Dioxide Combined
mm. Hg	*mm. Hg*	*vol. per cent*
air	21.48	13.42
air	21.50	11.50
air	19.85	10.90
air	23.90	11.21
3.54	23.62	11.85
3.70	23.65	11.50

tonometers used for the " reduced " samples would not oxygenate more than ten per cent of the hemoglobin. Disregarding the first experiment of the series, the combined carbon dioxide is the same within the limits of experimental error in both series.[2] Certainly the phenomenon does not occur with the magnitude commonly observed in the blood of the higher vertebrates, and as in the case of the skate, it may be concluded that the reciprocal effects of oxygen and carbon dioxide upon the equilibrium of *Urechis* hemoglobin with these gases are absent or nearly so.

[2] A second experiment in which the corpuscles were not so highly concentrated and in which the measurements did not agree so closely yielded higher CO_2 values for the oxygenated than for the reduced samples.

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The distribution of carbon dioxide between the cells and plasma is of particular interest as *Urechis* is the most primitive type of animal in which the respiratory properties of the corpuscles have been studied. The red blood corpuscle of *Urechis* is a much more " typical " cell than are those of the vertebrates and one looks for properties which contrast it with these more highly specialized erythrocytes.

TABLE IX

Data on Distribution of Carbon Dioxide between Corpuscles and Plasma

Specimen No.	19	22	24	25	26
Temperature of equilibration—° C.	19.5	19.0	20	19	19
Volume of corpuscles—*per cent*	23.0	34.5	23	34.7	31.7
Low Pressure Experiment					
CO_2 pressure of equilibration—*mm. Hg*	8.0	12.95	10.50	10.20	9.31
Whole blood-CO_2 content—*vol. per cent*	8.82	14.20	11.40	11.18	10.80
True plasma-CO_2 content—*vol. per cent*	7.09	14.15	11.30	10.95	11.45
Separated serum					
CO_2 pressure of equilibration—*mm. Hg*	53.6	68.4		54.7	47.0
CO_2 content at this pressure—*vol. per cent*	11.74	19.30		16.54	15.40
High Pressure Experiment					
CO_2 pressure of equilibration—*mm. Hg*	62.5	62.2		49.5	46.7
Whole blood-CO_2 content—*vol. per cent*	20.7	23.5		19.4	19.56
True plasma-CO_2 content—*vol. per cent*	18.9	20.4		17.12	18.82

Several experiments have been made in order to elucidate the respective parts which corpuscles and plasma play in the transport of carbon dioxide, and to determine the extent to which there is an exchange of material between cells and plasma. The procedure has been to equilibrate blood with carbon dioxide at a pressure comparable to that existing *in vivo*. With a part of this solution duplicate determinations were made of the carbon dioxide content of the *whole blood*. The remainder was centrifuged under oil in stoppered tubes. A portion of the plasma so separated was analyzed for the carbon dioxide content of the *true plasma, i.e.,* the plasma in equilibrium with corpuscles at the original carbon dioxide tension. From these measurements together with a measurement of the fraction of the whole blood occupied by the corpuscles, made by hematocrit, the ratio of carbon dioxide concentration in corpuscles and plasma could be calculated. The remainder of the plasma was then equilibrated with a relatively high CO_2 tension in order to give an idea of the buffer action of the *separated plasma*. The foregoing measurements, which are designated as the " low pressure experiment " in the tables, were performed in the mornings on which the blood was drawn. In the afternoon the " high pressure experiment " was carried out. A portion of the whole blood was now

equilibrated at a relatively high CO_2 pressure and its CO_2 content and that of the true plasma determined. The data of the experiments are given in Table IX and certain calculations based on these data appear in Table X. Referring to the latter, several definite conclusions may

TABLE X

Certain Indices of the Distribution of Carbon Dioxide between Corpuscles and Plasma

Specimen No.	19	22	24	25	26
Ratio of CO_2 concentration between corpuscles and plasma					
Low pressure experiments..............	(2.05)	1.01	1.04	1.03	0.82
High pressure experiments..............	1.43	1.43	—	1.38	1.29
Change in CO_2 content per unit change in CO_2 pressure					
Separated serum—*vol. per cent per mm. pressure*........................	0.102	0.093	—	0.125	0.105
True serum—*vol. per cent per mm. pressure*.	(0.217)	0.127	—	0.156	0.197
Whole blood—*vol. per cent per mm. pressure*	0.218	0.189	—	0.208	0.234
Total buffer value of blood; β...........	9.6	7.3		7.6	9.2
Volume of corpuscles—*per cent*..........	23.0	34.5	23.00	34.7	31.7

be drawn. In other regards the results of the experiments are at variance and the interesting question emerges as to whether the apparent variability in behavior may be due to the properties of the relatively unspecialized cell which serves as erythrocyte in *Urechis*.

The ratio of the CO_2 concentration of whole blood to that in the true plasma is approximately 1.0 in the low pressure experiments. The discordant value in Experiment No. 19 is probably to be attributed to experimental error. This means that CO_2 is about equally distributed between corpuscles and plasma under pressure conditions such as occur in the blood of the worm. The effects of the Donnan equilibrium and the corrections for the volume occupied by solutes in the corpuscles and plasma are neglected and would probably be too small to be significant in measurements as inaccurate as those employed.

The ratio of carbon dioxide concentration between cells and plasma in the high pressure experiments is uniformly greater than one. This result indicates that the principal buffer substances occur within the corpuscle and that the exchange of materials between corpuscles and plasma (the chloride shift) which enables the corpuscles to contribute to the buffer action of the plasma in the higher vertebrates is restricted in the case of the *Urechis* blood corpuscle. It has been shown above that the hemoglobin concentration is sufficient to account for the total buffer action of *Urechis* blood. The apparent restriction in the ex-

change of electrolytes between corpuscles and plasma is perhaps to be related to the tough membrane which may be observed to surround the erythrocyte of *Urechis*.

The relative part played by corpuscles and plasma in *Urechis* blood is further expressed by the estimation of the change in CO_2 content of the components per unit change in CO_2 pressure in passing from the low to the high pressure stages of the experiment. This method of expressing the results is somewhat arbitrary, as the relation is not strictly comparable for varying ranges of pressure. However, for the present purpose of comparing data made at two similar pressures considerably separated, it is convenient.

The increase in CO_2 content with increase of pressure in the case of the *plasma separated at low tensions* is fairly uniform and has an average value of 0.106 volumes per cent per millimeter pressure. This is almost exactly the rate of increase which would be due to the solution of carbon dioxide in the plasma if the absorption coefficient is 0.80 as assumed above. Parsons and Parsons (1923) publish some measurements of the carbon dioxide content of sea water at various CO_2 pressures from which it appears that the rate of increase is 0.109 volumes per cent per millimeter pressure. It is concluded that the plasma of *Urechis* contains at most a negligible quantity of buffer material, the increase in CO_2 content being adequately accounted for by the solubility of carbon dioxide.

Turning to the whole blood, the increase in CO_2 content with change in pressure is reasonably concordant in the various experiments and yields values about twice as great as in the case of the separated plasma. Between the pressures examined the gain in bound CO_2 is about equal to the gain in CO_2 dissolved.[3] This is a further expression of the fact that the corpuscular content is responsible for the buffer action of the blood. The buffer values for the whole blood recorded in Table X are slightly less than that estimated from the experiment recorded in Fig. 5. The variation in buffer value in different samples of blood does not appear to be closely correlated with the volume of cells in the samples. Presumably the variation in hemoglobin concentration in the cells from different specimens indicated in Table III is sufficient to destroy the expected correlation.

The true plasma shows a gain in CO_2 content with increasing CO_2 pressure which is variable but always less than the corresponding gain for whole blood and always greater than the gain shown by plasma separated at low pressures. The latter fact indicates that with increas-

[3] Over a shorter range the gain in bound CO_2 would be relatively greater than this because of the " shape " of the carbon dioxide-combining curve.

ing CO_2 pressure some exchange of material between corpuscles and plasma takes place which increases the ability of the plasma to take up carbon dioxide. This is presumably a " chloride shift " such as occurs in mammalian blood. With the exception of Experiment No. 19 the true plasma always gains less carbon dioxide than does whole blood. This is a further expression of the fact, brought out in the consideration of the ratio of carbon dioxide concentration in cells and plasma, that the exchange of materials affecting buffer action is limited. In Experiment No. 19 the high value of the carbon dioxide uptake of the true plasma is probably due to the experimental error which caused the ratio of carbon dioxide in cells and plasma to appear abnormal.

II. Physiological Observations

The Oxygen Content of the Blood in vivo

Samples of blood were drawn from worms lying in a pan of fresh sea water by inserting into the cœlomic cavity a hypodermic needle attached to a graduated one cc. pipette. The blood flowed into the pipette from its own pressure and was transferred directly into the Van Slyke apparatus for analysis.[4] Immediately following a larger sample of blood was drawn, equilibrated with air for 20 minutes and analyzed, thus yielding a measure of the oxygen capacity of the blood. Table XI

Table XI

The Oxygen Content of Urechis Blood in vivo

Experiment No.	In vivo		Saturated with Air	
	Temperature	Oxygen Content	Temperature	Oxygen Content
	° C.	volumes per cent	° C.	volumes per cent
9	15	2.87	18	2.85
			18	2.87
20	18.5	3.45	19	3.70
21	18.5	4.11	19.5	4.43
			19.5	4.50

contains the results of three such experiments. The figures are not corrected for dissolved oxygen. In Experiment No. 9 the oxygen content of the blood *in vivo* is equal to that of blood saturated with air.

[4] The pressure existing in the cœlomic fluid may be about sixteen grams per cm.², for on one occasion in drawing blood from the cœlomic cavity the blood rose in the pipette to a vertical distance of 16 cm. and oscillated about this level as the result of the muscular contractions of the body wall.

In the other two experiments the oxygen content is slightly less than the oxygen capacity. The result indicates that the pressure of oxygen in the blood may be considerably less than that of air. The diminished oxygen content in the blood *in vivo* is largely due to the smaller amount dissolved rather than to incomplete oxygenation of the hemoglobin. Thus, in the case of Experiment No. 21, if we assume the oxygen pressure to be 75 mm., the hemoglobin would be 97 per cent saturated. The combined oxygen would amount to 3.85 volumes per cent if the total combining capacity be taken as 3.96 volumes per cent. The dissolved oxygen would be 0.25 volumes per cent, making the total content of the

TABLE XII

The Carbon Dioxide Content of Urechis Blood in vivo

Specimen No.	In vivo		In vitro		
	Temperature	CO₂ Content	O₂ Content†	CO₂ Pressure	CO₂ Content
	° C.	vol. per cent	vol. per cent	mm. Hg	vol. per cent
10	15	7.12	5.62		
11	15.5	8.12	4.65	10.4*	10.67
				36.0*	18.95
12	16.5	8.79	4.4	See Table VII	

* Equilibrated at 18° C.
† Equilibrated with air.   Not corrected for dissolved oxygen.

blood 4.10 volumes per cent as observed. It is concluded that the hemoglobin of *Urechis* is almost completely saturated when an abundant supply of oxygenated water is available for respiratory purposes, but that the pressure of oxygen in the blood may be considerably lower than that existing in the water.

The Carbon Dioxide Content of the Blood in vivo

Table XII contains data on the carbon dioxide content of the blood *in vivo* obtained in a manner similar to that of the oxygen capacity. The measurements indicate a normal carbon dioxide content of between seven and nine volumes per cent. Data for the equilibrium of carbon dioxide with the blood used in Experiment No. 12 are recorded in Table VII and Fig. 5. Similar data for two points on the CO₂ dissociation curve of the blood used in Experiment No. 11 are included in Table XII. These data agree closely with those in Table VII. From Table VII it appears that at the CO₂ content observed *in vivo*, 8.79 volumes per cent, the pressure of carbon dioxide would be approximately 7.2 millimeters.

The pH Value of Urechis Blood in vivo

The pH value of blood plasma is given by the equation

$$pH = pK' + \log \frac{(BHCO_3)}{(H_2CO_3)},$$

in which pK' is a constant dependant upon the properties of the corpuscles and plasma. The value of pK' for *Urechis* blood is unknown, but it cannot differ greatly from 6.1 when the corrections for temperature (Warburg, 1922), ionic strength (Hastings and Sendroy, 1925) and corpuscular content (Van Slyke, Hastings, Murray and Sendroy, 1925) are taken into account. Since the value of $\log \frac{(BHCO_3)}{(H_2CO_3)}$ for *Urechis* blood at 7.22 mm. is about one, the pH value of the blood plasma *in vivo* must be close to 7.1. The blood is therefore somewhat more acid than human blood and much more acid than sea water. The value of $\log \frac{(BHCO_3)}{(H_2CO_3)}$ indicates that one eleventh of the carbon dioxide of the blood is in solution, the remainder being bound as bicarbonate.

The Exchange of Gas between Blood and Sea Water

Urechis lives in a permanent burrow in flats which are occasionally exposed at low tide. The burrows are U-shaped, having two openings. Water is circulated through the burrow by means of peristaltic contractions of the body wall, which force the fluid backward between the worm and the wall of the tube. The flow thus established serves both for respiration and to bring the animal its food supply. The worms may be kept for long periods in the laboratory confined in artificial burrows constructed of glass tubing, and under these conditions the volume of water circulated and the changes in its gaseous content may be measured. The respiratory and feeding reactions of animals so confined are fully described by Fisher and MacGinitie (1928). They consider that respiration is principally effected by means of water pumped into the hind-gut, through the activity of the muscular cloaca. The structure of the hind-gut as well as the active rhythm through which it is ventilated certainly support this view.

The hind-gut is a large sack extending the length of the body and occupying the greater part of the cœlomic cavity. Its wall is smooth and so thin as to be quite translucent, resembling in this regard a mesenteric membrane. The wall of the hind-gut is bathed directly by the blood, there being no blood vessels (Fisher and MacGinitie, 1928). The peristaltic movements of the body wall must produce some circulation in the blood. Rhythmic contractions of the hind-gut wall appear

to be important in bringing the blood into contact with the hind-gut wall as well as in mixing the water in the hind-gut. If the worm is examined against the light one may see the outlines of the hind-gut, which appears as a relatively transparent region. It may be observed that the hind-gut is the seat of antiperistaltic contractions which sweep over it in the form of deep annular constrictions into which the blood is drawn and carried along. Compared to this effective mechanism the thick, muscular, cuticulated body wall must absorb a relatively small amount of oxygen.

The water within the hind-gut is renewed by a somewhat irregular rhythm. Fresh water is drawn in by a series of from one to upward of thirty small inhalations usually uninterrupted by exhalation. It is then discharged by means of a single exhalation, frequently followed by a period of rest. Fisher and MacGinitie record periods of inhalation lasting from twenty-five to ninety seconds and expirations consuming from ten to fifty seconds.

Samples of hind-gut water have been collected at the moment of expiration. The worm is watched through the glass wall of the aquarium until it begins to discharge the water in a vigorous stream which may be easily observed. At that moment the worm is taken out of the aquarium and the anal end thrust tightly into a funnel terminating under oil in a suitable glass-stoppered bottle. The worm continues to discharge the hind-gut water, which is collected under the oil. From 25 to 35 cc. of water may be obtained at a single discharge. Several discharges were combined to yield material for oxygen analysis by the Winkler method. Carbon dioxide content has been determined with the Van Slyke apparatus. Table XIII contains the results of a number of such determinations, together with control measurements made upon the sea water of the aquarium.

TABLE XIII

Oxygen and Carbon Dioxide Content of Hind-gut Water

Oxygen

Hind-gut water............0.37, 0.37, 0.29, 0.36, 0.36, 0.37 vol. per cent
Aquarium water............0.56, 0.56   vol. per cent

Carbon Dioxide

Hind-gut water............5.26, 5.11 vol. per cent
Aquarium water............4.77, 4.79 vol. per cent

The oxygen content of the expired water is about two-thirds that of the sea water. The partial pressure of oxygen in the hind-gut water is thus about one hundred millimeters and is quite sufficient to account for the high degree of saturation found in the blood *in vivo*.

The carbon dioxide measurements may be evaluated by means of

determinations made by Parsons and Parsons (1923) of the carbon dioxide content of sea water from the Naples aquarium at various pressures. They found at 0.8 mm. CO_2 pressure a content of 4.7 volumes per cent which agrees closely with the values found in our aquaria. Interpolating from their data the carbon dioxide pressure of the expired water corresponds to 4.6 and 6.0 mm. in the two samples examined. Since the partial pressure of CO_2 in the blood is about seven millimeters, a gradient of pressure of about two millimeters occurs across the hind-gut wall.

From the foregoing experiments certain deductions may be drawn relative to the volume of water necessary to " ventilate " the hind-gut. Dr. V. E. Hall, who has been engaged in a study of the respiratory and feeding reactions of *Urechis*, has kindly supplied data concerning the volume of water pumped by *Urechis* through artificial burrows made from glass tubing, and the rate of oxygen consumption of the worms. The average rate of oxygen consumption of two medium-sized *Urechis* was about 0.013 cc. per minute. The amount of water pumped when the worms were not engaged in feeding was about 11 cc. per minute; when feeding, it was about 29 cc. per minute. There is required 2.3 cc. of sea water containing 0.56 volumes per cent oxygen to yield the 0.013 cc. consumed in one minute. When the water is expired from the hind-gut only one-third of the oxygen dissolved in it has been consumed. Consequently 6.9 cc. of water must ventilate the gut each minute. This is about half the amount pumped through the burrows when feeding is not going on. Feeding worms pump about four times the required volume of water, but under these circumstances the water is serving to bring food to the animal as well as for respiration. The size of the animals and their activity are variable and consequently these estimations cannot be very exact. They show, however, that the respiratory activity of the animal is rather nicely adjusted to the metabolic requirements.

The Function of the Hemoglobin of Urechis

The data in Table XI show that the hemoglobin of *Urechis* is almost completely saturated when an abundant supply of aërated water is available to the animals. The preceding considerations indicate that the mechanisms for bringing fresh water into contact with the respiratory surface of the hind-gut operate with a fair margin of safety at each step. Under ordinary conditions it appears that the oxygen bound to the hemoglobin is not utilized and that the oxygen dissolved in the plasma is sufficient for the metabolic requirements. *Urechis* must be added to the list of animals, including *Planorbis* (Leitch, 1916) and *Lumbricus* (Jordan and Schwarz, 1920), in which the hemoglobin does not appear to function if the oxygen supply is adequate.

Light is thrown on the possible value of the hemoglobin to the worms by considering the rate at which oxygen " circulates " through the blood. The problem is somewhat less definite in *Urechis* than in the vertebrates because there are no blood vessels and the ordinary conceptions of arterial, capillary and venous blood do not apply. If we consider 20 cc. to be the blood volume of *Urechis* and four volumes per cent to represent the oxygen capacity, then the oxygen content of the total blood is 0.8 cubic centimeters. Taking the rate of oxygen consumption to be 0.013 cc. per minute, it follows that only one-sixtieth of the oxygen content of the blood is used (and need be replaced) per minute. It is clear from this why the *Urechis* blood is almost completely saturated *in vivo*. It also follows that those properties which assist mammalian blood to give off or take up oxygen and carbon dioxide rapidly during its passage through the capillaries (the reciprocal action of oxygen and carbon dioxide on the equilibrium of these gases with hemoglobin and the transfer of buffer action from cells to plasma) may be dispensed with in *Urechis* blood.

The blood of *Urechis* appears from the foregoing observations to contain a store of oxygen sufficient to last the animal one hour. In addition the hind-gut water itself, having a volume of about thirty cc., contains some 0.11 cc. oxygen. This would serve to supply the metabolic requirement for not more than 8.5 minutes. The total oxygen within the animal consequently is enough to last about seventy minutes.

Consider what would happen if the blood contained no hemoglobin. In it the oxygen concentration would be no greater than in the hind-gut water, say 0.37 volumes per cent. The total volume of oxygen in 20 cc. of blood would be .074 cubic centimeters. At a metabolic rate of 0.013 cc. per minute this would last the animal 5.7 minutes. Adding to this the time which the oxygen in the hind-gut would serve, the total oxygen within an animal without hemoglobin would last about fourteen minutes.

The hemoglobin of *Urechis* consequently extends the period during which the respiratory exchange might be interrupted without depriving the animal of oxygen about five-fold, or for about fifty-five minutes. This is not long enough to carry the animal over the period of a low tide, when the burrows are exposed. It is sufficient to be useful during the " rest periods " which occur after a more or less prolonged period of feeding. According to Fisher and MacGinitie (1928), these rest periods are of two sorts: (1) intermittent periods of from 4.5 to 8.5 minutes separated by about 1.5-minute intervals, during which water is expelled from the respiratory chamber and a new supply taken, (2) a continuous rest of an hour or more during which respiration ceases (or at least is so reduced as to be imperceptible) and no movement of any kind takes place.

The Oxygen Supply When the Tide is Out

On the California coast the tides follow a rhythm in which alternate tides are of unequal height. The low course tides are more nearly equal and in the estuary where *Urechis* was found the flats are not uncovered. During the high course tides the flats are uncovered once a day for a period of six or more hours. During the greatest spring tides the estuary sometimes empties so completely during the lower ebb tides that it does not fill during the succeeding flood tide and in consequence the flats may be bare for 18 hours.

During the period when the tide is out there is available for the worms not only the oxygen in the blood and hind-gut water, which we have seen is adequate for the metabolic requirements for about seventy minutes, but also the oxygen dissolved in the water enclosed in the burrow. An average burrow is about one hundred centimeters long and two centimeters in diameter. It would contain some 314 cc. of water, and if this were saturated with air about 1.76 cc. of oxygen. This would last 135 minutes if used at a rate of 0.013 cc. per minute. The total oxygen supply of *Urechis* during low tide is sufficient for only about three hours according to these calculations.

In order to throw more certain light on the state of affairs during low tide, a series of analyses on the oxygen content of the water in the burrows was made. A rubber tube was thrust down into the burrows and sufficient water for analysis by the Winkler method (70 cc.) drawn out and transferred to a glass-stoppered bottle without exposure to air. ∴ new burrow was selected for each observation. The flats had already become bare when we arrived but had not been so for more than one-half hour to judge from the state of the tide when the first observation was made. The last observations were made from the last burrows to be covered after the greater part of the flat was submerged by the rising tide. The oxygen content of the water left in a puddle by the receding tide, which serves to give an idea of the content of the burrow water before the flat was bared, was 0.34 volumes per cent. This relatively low value may be accounted for by the fact that the observations were made at daybreak. The water had been overnight in an estuary teeming with animal and vegetable life and oxygen losses had not been compensated by photosynthesis. The temperature of the water in the burrows was $15°$ C. at 6:08 A.M. and had risen to $17°$ C. at 11:30. At this time the water in the channel was $19°$ C. The results are recorded in Table XIV.

During the first hour after the flat is bare the oxygen content of the burrow water appears to decrease rapidly and at about the rate expected from the foregoing calculations. There is some irregularity in the

TABLE XIV

Oxygen Content of Water from Urechis Burrows During Low Tide

Time—Samples Collected	Approximate Time Since Flat Became Bare	Oxygen Content of Water	
		From Individual Burrows	Mean
	hours	*cc. per 100 cc.*	*cc. per 100 cc.*
6:08 A.M.	0.5	0.21	0.21
6:24	0.7	0.16	0.16
6:30	0.9	0.13	0.13
6:35	1.0	0.15; 0.11; 0.11	0.12
7:40	2.0	0.24	0.24
7:55	2.2	0.16	0.16
8:00	2.5	0.12	0.12
9:30	4.0	0.06; 0.06; 0.06	0.06
10:30	5.0	0.16; 0.14; 0.14	0.14
11:15	5.7	0.06; 0.16; 0.23; 0.27; 0.27	0.20

individual measurements made during the third hour, but by the fourth hour the oxygen content has definitely sunk to a minimal value of 0.06 volumes per cent. During the fifth hour there is a perfectly definite increase in the oxygen content of the water in almost all of the burrows examined. These measurements support the view that the oxygen in the water inclosed within the burrow and in the blood is insufficient to maintain the normal metabolic rate for the duration of the low tide. After the first hour the oxygen in the burrow water diminishes rather slowly and one must conclude, either that the rate of oxygen consumption by the worm is diminished or that the oxygen in the burrows is replenished by some means. There is reason to believe that both these processes occur. It is well established that the metabolic rate of many marine organisms varies with the oxygen pressure in the environment (Amberson, Meyerson and Scott, 1924; Hall, 1929, and others). The measurements made on the water of the burrows very definitely indicate an increase in the oxygen content during the last hour before the flats were covered. This suggests that the water in the burrows is slowly replaced by the water with which the sand is permeated. The effect is probably related to changes of hydrostatic pressure within the flat occasioned by changes in the tide level, for it is reported that wells in sandy soil near the sea sometimes display definite changes in level related to the tides. The effect becomes noticeable only during the last hour when the tide is rising rapidly. It is presumably occurring throughout the low tide period and serves to check the exhaustion of the oxygen content of the water by the metabolism of the worms. If this view is correct it serves to explain how *Urechis* can withstand the 18 hours of

low water which occur during the spring tides. At these times the small intermediate tide, although unable to cover the flats, will serve to move about the water within the flats and thus replenish to a certain degree the oxygen within the burrows of *Urechis.*

The oxygen content within the burrow never appeared less than 0.06 volumes per cent, which corresponds to an oxygen pressure of about fourteen millimeters. At this pressure the hemoglobin of *Urechis* is nearly 60 per cent saturated. During the greater part of the low tide the pressure of oxygen in the burrow is such that the hemoglobin of the blood will function effectively as an oxygen carrier while very little oxygen will be present in solution in the blood. Provided the oxygen in the burrows does not sink below the observed levels, the hemoglobin of the blood may be expected to transport an adequate supply of this gas to the organs of the body.

SUMMARY

1. The blood of *Urechis caupo* contains hemoglobin enclosed in corpuscles. The oxygen capacity of the blood varies from 2.66 to 7.22 volumes per cent and the percentage of cells in the blood from 18 to 40.

2. The oxygen dissociation curve is measured. Its position does not appear to be influenced by the carbon dioxide pressure. The effect of temperature upon the oxygen dissociation curve is of the direction and order observed in other bloods containing hemoglobins.

3. The carbon dioxide dissociation curve is measured. The ability of the blood to combine with carbon dioxide does not appear to be influenced by the degree of oxygenation of the blood.

4. The buffer value of the blood is 11 volumes per cent and is constant over a considerable range of carbon dioxide pressures. The concentration of hemoglobin accounts for the entire buffer effect.

5. Carbon dioxide is about equally distributed (in concentration) between the corpuscles and plasma. The plasma contains at most a negligible quantity of buffer material. With increased carbon dioxide tension there is a small, but distinctly limited exchange of material between the corpuscles and plasma which increase the ability of the latter to combine with carbonic acid.

6. The hemoglobin *in vivo* is almost completely saturated, but the pressure of oxygen in the blood may be considerably less than that in the surrounding water. The carbon dioxide content *in vivo* is 7 and 9 volumes per cent, corresponding to a carbon dioxide pressure of about seven millimeters Hg. The reaction of the blood is estimated to be about pH 7.1.

7. The " ventilation " of the respiratory organ, the hind-gut, is
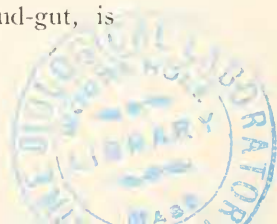
considered quantitatively, the result indicating that the respiratory activity is nicely adjusted to the metabolic requirements.

8. The function of hemoglobin and its relation to the oxygen supply during low tide are discussed. It is suggested that the movement of water within the flats due to changing tidal level is important in supplying oxygen when the tide is out.

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